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Solution Structure and Interdomain Interactions of the Saccharomyces Cerevisiae TATA Binding Protein Probed by Radiolytic Protein Footprinting

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The selection of the RNA polymerase by which genes in eukaryotic organisms are transcribed is the result of the assembly on promoters of proteins called “transcription factors” that are specific to each of the three RNA polymerases. The ‘TATA Box Binding Protein’ (TBP), is a component of the nucleo-protein complexes required for the initiation of transcription by each of the three eukaryotic RNA polymerases. TBP binds with high affinity and specificity DNA with a consensus sequence of TATAa/tAa/t. TBP introduces a dramatic and unusual bend to the bound DNA that is TATA sequence and solution condition dependent. Although atomic resolution crystal structures of the conserved C-terminal domain of several species of TBP and their complexes with DNA have been determined, little information is available concerning the structure in solution of full length TBP containing both the conserved C-terminal and nonconserved N-terminal domains.

Quantitation of the amino acid side chain oxidation products generated by synchrotron X-ray radiolysis by mass spectrometry has been used to determine the solvent accessibility of individual residues in monomeric *Saccharomyces cerevisiae* TATA Binding Protein (TBP) free in solution and in the TBP-DNA complex. Amino acid side chains within the C-terminal domain of unliganded TBP that are predicted to be accessible from crystal structures of the isolated domain are protected from oxidation. Residues within the N-terminal domain are also protected from oxidation in both the absence and presence of DNA. While residues within the DNA-binding ‘saddle’ of the C-terminal domain are protected upon formation of a TBP-DNA complex as expected, residues on the upper side of the beta-sheets, also undergo reactivity changes. These data suggest that the DNA-binding saddle of monomeric unliganded yeast TBP is only partially accessible to solvent the N-terminal domain is partially structured and the N- and C-terminal domains form a different set of contacts in the free and DNA-bound protein.